Toxicity of Dialyzed Extracts of Some California Anemones (Coelenterata)[1]

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ABSTRACT: Anemones of the California coast, collected from November 1960 to March 1961, were compared with respect to their toxicity. Aqueous extracts of the anemones were dialyzed and assayed by intraperitoneal injection to mice, and the survival times of the injected mice were compared.

The extracts of *Anthopleura elegantissima* and *A. xanthogrammica* were by far more toxic than those of *Metridium senile, Corynactis californica, Tealia crassicornis, T. lofotensis,* and *T. coriacea.*

The differences in potencies of the extracts may indicate species variations in (1) toxicity, (2) efficiency of the extraction method, or (3) both.

THE AQUEOUS EXTRACTS of many coelenterates are poisonous (Dodge, 1957). The poisons are mixtures of biologically active compounds (Crescitelli and Geissman, 1962), some of them amines (Welsh and Prock, 1958), others heat-labile colloids (Martin, 1960; Mathias et al., 1960). The poison occurs in both the nematocysts and the nematocyst-free structures of the anemones (Lane, 1960; Martin, to be published). Both the chemical composition and the biologically active substances of extracts vary from one species of anemone to another (Mathias et al., 1960; Bergmann et al., 1956). The present study was designed to compare the lethality in mice of dialyzed extracts of some anemones of the California coast.

MATERIAL AND METHODS

SPECIES AND SITES OF COLLECTION: Anemones were collected at various sites on the California coast from November 1960 to March 1961: *Anthopleura elegantissima* from Bodega Bay, Tomales Bay, Monterey, and Corona del Mar; *A. xanthogrammica* from Tomales Bay and Monterey; *Metridium senile* from Monterey; *Corynactis californica, Tealia lofotensis* and *T. crassicornis* from Bodega Bay, all intertidal; and *T. coriacea* off Marineland from a 30-ft depth. In addition, the intertidal Nudibranch *Diaulula sandiegensis* and the Mollusca *Crassostrea gigas* and *Mytilus californianus* were collected at Tomales Bay. *Anthopleura elegantissima* is common all along the California coast. Its extracts were used as the base of comparison with those of the other species available.

The anemones were placed in the laboratory in aerated aquaria, with sea water which was renewed twice a week. Water temperature varied between 13 and 20 C. The anemones were exposed to the light of the laboratory. They received no food supplement. Specimens which did not show normal vitality in the aquaria were discarded. After 2–4 weeks the anemones were removed from the aquaria and cleaned from adherent objects. During this manipulation they contracted firmly. Their body wall was then punctured to empty pockets of trapped water. Then the anemones were rinsed with distilled water for 10–15 sec, blotted with mild pressure, weighed in air, and their volume determined. The specimens weighed from 0.3 to 34.2 g.

EXTRACTION: The anemones were homogenized with three times their volume of distilled water at high speed in a Waring blender for

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two 1-min periods. After being centrifuged for 30 min at 1000 g the supernatant was decanted and centrifuged again. The resulting supernatant extract was turbid but did not contain cells or debris. It was either decanted or, when it was found covered with a lipid layer, removed with syringe and needle; then its pH was determined. The extract was dialyzed for 4 hr through commercial cellophane tubing against ten times its volume of 0.001 M sodium phosphate (pH 6.2) in 0.8% sodium chloride with 1% activated charcoal (Weinberger, 1936). The dialysis was repeated once with fresh solution. All operations were performed at room temperature.

Extracts of the other lower metazoa were prepared in the same manner. All extracts were assayed immediately after their preparation. The potency of extracts of each species was compared with that of an extract of *A. elegantissima* prepared on the same day.

ASSAY: The extract was given intraperitoneally to DAL-Swiss albino mice weighing 17 to 23 g. The mice were placed in cages in lots of four to eight (Russell et al., 1960), and observed for 24 hr; their survival time was recorded. The abdominal cavities of the mice were inspected post mortem and mice with intraperitoneal hemorrhage were discarded. This condition was found in animals that showed severe pain reaction immediately after the injection and died within 4 min. The discarded animals were replaced by supplementary ones to complete the series. Each extract was assayed on a series of at least four mice.

EXPERIMENTS AND RESULTS

Preliminary experiments explored the effect of varying doses of extracts of *A. elegantissima* on the survival time of the injected mice. Only the area of LD_{99} was considered. A dose effect curve showed that with decreasing doses the survival time increased. At the dose of 20 cc of extract per kilo of mouse, both the range and the mean of the survival time were within practical limits; therefore, this dose was selected as a convenient reference.

The pH of all the extracts varied from 6.0 to 6.6.

At a dose of 20 cc of extract of *A. elegantissima* per kilo of mouse, the mean survival time of the injected mice varied from 8 to 36 min among the ten series tested. Between some of these series the difference in survival time was significant at the 2% level by the Chi-square test. The cause of these differences is unknown. The differences showed no correlation with variations in the size, sites of origin, or dates of collection of the specimens, or with the pH of the extracts. They were not relevant for the present study.

Table 1 shows that the potency of extracts may vary from one species of anemone to another. The highest potency, as estimated by the survival time of mice after intraperitoneal injection, was found for the extracts of *A. elegantissima* and *A. xanthogrammica.* Extracts of other anemones were far less potent even when their dose was tripled. With extracts of *T. lofo-*

TABLE 1

COMPARISON OF THE SURVIVAL TIME OF MICE AFTER INTRAPERITONEAL INJECTION OF EXTRACTS OF ANEMONES

SPECIES	NO. OF BATCHES	NO. OF MICE	DOSES CC OF EXTRACT/KG OF MOUSE	SURVIVAL TIME IN MINUTES		PROPORTION OF MICE SURVIVING 24 HR
				Mean	Range	
Anthopleura elegantissima	10	48	20	15.7	5–47	1/48
A. xanthogrammica	2	10	20	9.2	6–15	0/10
Metridium senile	1	6	20	174.1	80–240	0/6
Corynactis californica	1	5	60	138.5	118–180	0/5
Tealia crassicornis	1	6	60	342.8	252–480	0/6
T. lofotensis	1	4	20		336	3/4
T. lofotensis	1	4	60		460–540	2/4
T. coriacea	1	4	20		264–640	2/4
T. coriacea	1	4	60		480–900	2/4

tensis and *T. coriacea* it was not possible to obtain an LD_{99}.

The extracts of the Nudibranch *Diaulula sandiegensis* and the Mollusca *Crassostrea gigas* and *Mytilus californianus,* each assayed on six mice at the dose of 60 cc extract per kilo of mouse, caused transitory depression of activity of the injected mice, but not death.

The extracts of all anemone species lost their toxicity when they were heated at 90 C for 20 min.

DISCUSSION

The storing of the anemones alive for 2 weeks or longer before preparing the extracts was intended to minimize the amount of possibly toxic products from food ingested by them. The dialysis of the extracts was aimed at minimizing their content of biologically active amines. The nature of the non-dialyzable toxins is not known. They may be proteins similar to those demonstrated by various authors in other coelenterates (Mathias et al., 1960; Farber et al., 1961).

We found information concerning the chemical components of the anemones here investigated only in the literature on *A. elegantissima* (Bergmann and Landowne, 1958). In general the lipid content of coelenterates is high, and the proportions of various lipids and soaps and their chemical nature vary from one species to another (Bergmann et al., 1956; Bergmann and Landowne, 1958). The solubility of proteins is affected by lipids, and if it is assumed that the poisons here studied are proteins, it can be speculated that the solubility of these poisons may vary from one species to another.

The data of Table 1 suggest that the toxicity of anemones may vary from species to species. This interpretation is supported by the great difference between species with respect to potency of extracts, which was observed even when the dose of the weaker extracts was tripled. But it is also possible that the efficiency of the extracting method, and hence the poison content of the extracts, may vary from species to species. This consideration applies equally to our study and to other authors' reports of species variation in toxic compounds. These causes for variation may coexist.

Lethal effects on mice were observed with extracts of the anemones but not with extracts of the Nudibranch and the two bivalves. This suggests that toxicity is not a property of all littoral lower metazoa.

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